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Brief Communication

Diet and methyl mercury contamination of nestling red-winged blackbirds

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Abstract

Methyl mercury (MeHg) is a widespread environmental contaminant that can have adverse effects on the health of vertebrate wildlife. This study focused on diets and MeHg contamination of nestling red-winged blackbirds (*Agelaius phoeniceus*) from a wetland in north Texas, USA. In previous research at the study site, a risk assessment model suggested that if emergent aquatic insects (i.e., odonates) were the dominant prey item in nestling diets, the health of nestling red-winged blackbirds could be negatively affected. The purpose of this study was to follow up on an earlier risk assessment and determine whether nestling red-winged blackbirds were accumulating elevated concentrations of MeHg at our study site. We had four objectives: (1) estimate the proportion of emergent aquatic insects, spiders, and terrestrial insects in diets of nestling red-winged blackbirds using a stable isotope-based dietary mixing model; (2) assess the concentrations of MeHg in emergent aquatic insects, spiders, and terrestrial insects; (3) assess the concentrations of MeHg in blood of nestling red-winged blackbirds; and (4) determine whether nestling red-winged blackbirds had MeHg concentrations that were high enough to pose a health risk. We found that nestling red-winged blackbirds had a diet dominated by terrestrial insect prey with low concentrations of MeHg and that the nestlings had low concentrations of MeHg in their blood, well below hazardous levels. The results of the study suggest that caution must be used when interpreting risk assessment models for nestling red-winged blackbirds. Because their diets can consist of varying proportions of emergent aquatic insects, spiders, and terrestrial insects, risk assessments based on estimates of diet from the literature that suggest nestlings could be at risk from Hg contamination should be followed up with studies to assess diet and/or the actual level of contamination of nestlings.

Keywords: nestling red-winged blackbirds, mercury, food web, trophic transfer, stable isotopes

Introduction

Methyl mercury (MeHg) is a widespread environmental contaminant (Driscoll et al., 2013) that can have adverse effects on the health of vertebrate wildlife (Ackerman et al., 2016; Lepak et al., 2016). Historically, most studies focused on MeHg contamination of fish and fish-eating wildlife because MeHg is primarily formed in aquatic systems (Wiener et al., 2003). However, more recent studies have demonstrated that emergent aquatic insects, such as dragonflies and damselflies (i.e., odonates), transport MeHg out of aquatic systems and can expose terrestrial predators, such as birds, to MeHg (Brasso & Cristol, 2008; Chumchal & Drenner, 2020; Jackson et al., 2021). Riparian spiders that consume emergent aquatic insects also accumulate relatively high concentrations of MeHg in their tissues (Ortega-Rodriguez et al., 2019; Speir et al., 2014) and in turn expose songbirds that consume spiders to MeHg (Cristol et al., 2008; Gann et al., 2015). Conversely, taxa that feed primarily within food webs based on terrestrial primary production, such as terrestrial herbivorous insects, accumulate relatively low concentrations of MeHg, and the consumption of terrestrial insects by songbirds would result in a relatively low exposure to MeHg (Jackson et al., 2021; Ortega-Rodriguez et al., 2019; Speir et al., 2014).

This study focused on the diets and MeHg contamination of nestling red-winged blackbirds (*Agelaius phoeniceus*) from a wetland in north Texas, USA (Figure 1). Red-winged blackbirds are one of the most common birds throughout North America (Yasukawa & Searcy, 2020) and adults feed nestling red-winged blackbirds a variety of arthropod prey, which can include emergent aquatic insects, spiders, and terrestrial insects (online supplementary material Table S1). Although the proportion of arthropod prey items in nestling diets varies by study site, emergent aquatic insects can dominate nestling diet (online supplementary material Table S1).

In previous research at the study site, we determined that the nesting period of red-winged blackbirds overlapped with peak dragonfly and damselfly emergence (Williams et al., 2017). The concentrations of MeHg in odonates at the study site were relatively high, and a risk assessment model suggested that if odonates were the dominant prey item in nestling diets, the health

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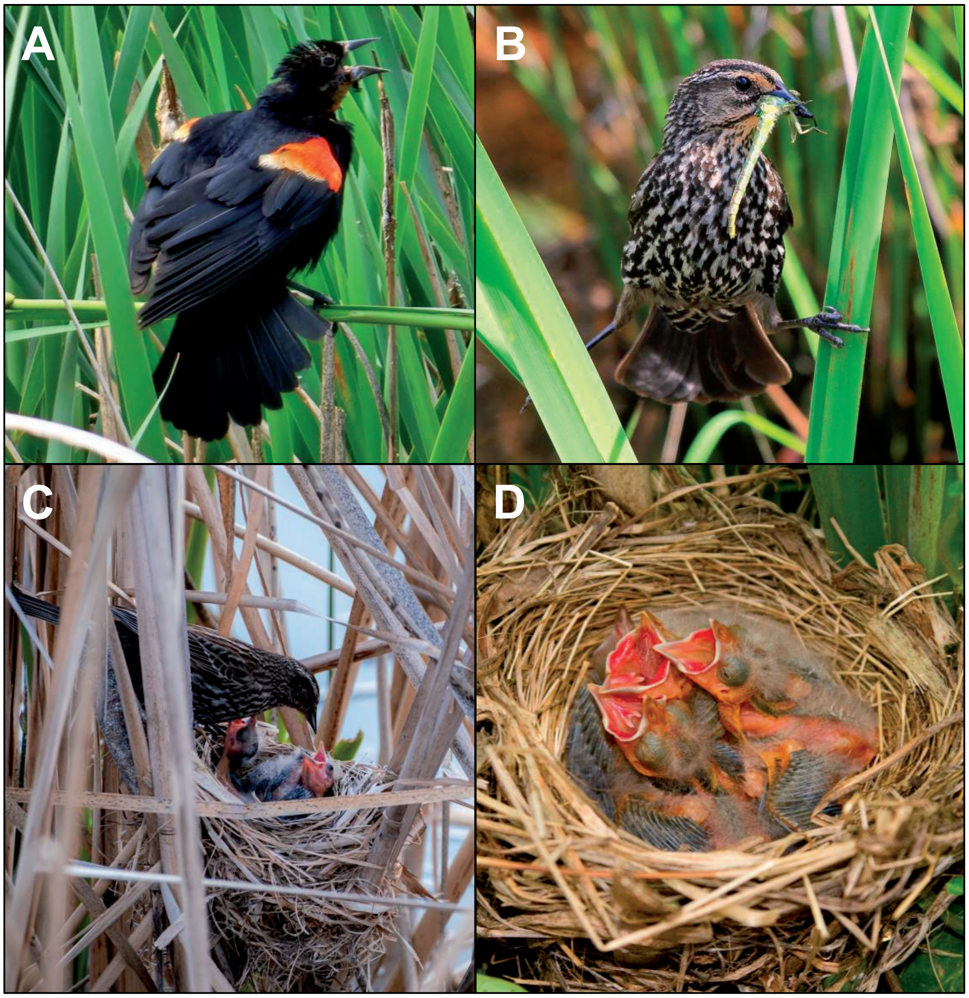

Figure 1. Male red-winged blackbirds (A) have conspicuous wing coloration and aggressively defend territories, whereas females (B) lack bright coloration. Red-winged blackbirds often nest in wetlands and build nests in emergent vegetation, like cattails (C). Red-winged blackbirds typically produce 2–4 nestlings per nest (D, Yasukawa & Searcy, 2020). The egg incubation period is 11–13 days and the nestling period is 11–14 days (Yasukawa & Searcy, 2020). Photo credits: (A) WhenJen, Shutterstock; (B) David Byron Keener, Shutterstock; (C) Christa Kartika, Shutterstock; (D) Marie Read, Alamy Stock Photo.

of nestling red-winged blackbirds could be negatively affected (Williams et al., 2017). The purpose of this study was to follow up on the results of our previous study (Williams et al., 2017) and determine whether nestling red-winged blackbirds were accumulating elevated concentrations of MeHg at our study site.

In the study, we had four objectives: (1) estimate the proportion of emergent aquatic insects, spiders, and terrestrial insects in diets of nestling red-winged blackbirds using a stable isotope-based dietary mixing model; (2) assess the concentrations of MeHg in emergent aquatic insects, spiders, and terrestrial insects; (3) assess the concentrations of MeHg in blood of nestling red-winged blackbirds; and (4) determine whether nestling red-winged blackbirds had MeHg concentrations that were high enough to pose a health risk.

Materials and methods

Study site

We conducted the present study at the Eagle Mountain Fish Hatchery (32°52′32.95″ N, 97°28′29.00″ W) near Fort Worth, Texas, USA (Figure 2). The site consists of ponds that are filled with water, dry ponds that formerly held water but have now been colonized with terrestrial vegetation (primarily grasses), and other terrestrial habitats that vary in landcover/land use (e.g., grass-covered levees, riparian forest, roads); hereafter, dry ponds are considered terrestrial habitat (Figure 2). Water is supplied to the ponds from the limnetic zone of Eagle Mountain Lake, a large drinking water supply reservoir. Ponds range in size from 0.23 to 0.54 ha with a mean depth of 0.8 m and are whole ecosystems with earthen bottoms that contain complex communities of macrophytes (including cattails [*Typha* spp.] and bulrush [*Scirpus* spp.]), benthic invertebrates, and some contain fish.

Ponds are contaminated with Hg originating from atmospheric deposition directly to the pond surface or the watershed of Eagle Mountain Lake (http://nadp.slh.wisc.edu). We are not aware of any point sources of Hg in the watershed. Previous studies revealed that aquatic insects emerging from ponds at the study site have ~5–40× higher concentrations of MeHg than terrestrial insects (Chumchal et al., 2017, 2018, 2022; Ortega-Rodriguez et al., 2019; Speir et al., 2014; Tweedy et al., 2013; Williams et al., 2017). Concentrations of Hg in spiders from the study site can be elevated relative to aquatic and terrestrial insects and are positively correlated with the proportion of emergent aquatic insects in their diets (Ortega-Rodriguez et al., 2019).

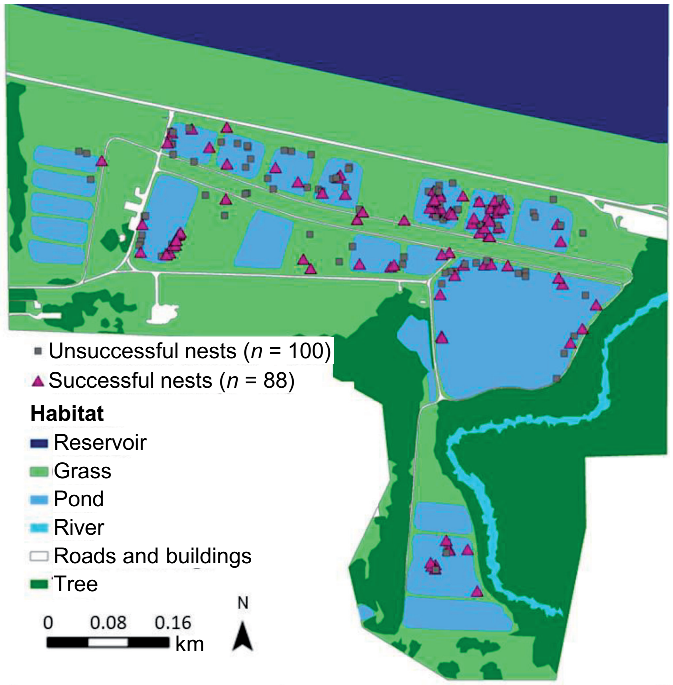

Figure 2. Map of the study area, active nests (nests being built or occupied with adults exhibiting defensive behaviors and/or that contained eggs or nestlings), and habitat. Successful nests are defined as nests where nestlings were sampled.

Monitoring of the study site for active red-winged blackbird nests

We began regularly monitoring the study site for active red-winged blackbird nests (defined as nests being built, occupied by adults exhibiting defensive behaviors, or containing eggs or nestlings) on January 26, 2017. After we observed nests being built in early April, we searched the study site every 1–3 days for active nests. Red-winged blackbird nests were located using systematic searches through emergent aquatic vegetation (both within and surrounding ponds) and terrestrial habitat. Typically, we identified nests by following adult birds to nests after observing parental behavioral cues (e.g., females carrying nesting material, defensive behavior). Global Positioning System coordinates of nest locations were recorded with the ESRI Collector for ArcGIS (Classic) phone application, which has a spatial resolution of 5 m. Once identified, active nests were monitored approximately every 2 days until hatch, which allowed us to determine nestling age. In some cases ($n = 15$ nests, 17%), we located nests after birds had already hatched and used leg width (measured using a banding gauge) and other traits (e.g., eyes closed vs. open, feather growth) to estimate age within 1–2 days.

From April 12 to July 31 (online supplementary material Figure S1), we identified 188 active nests, of which 88 nests (46% of those initially located) produced 1–4 nestlings (Figure 2). Potential foraging areas within a 100 m radius of each nest consisted of aquatic (40.5 ± 1.6% [mean ± *SE*]), terrestrial (54.3 ± 1.4%) and human-built habitats (5.2 ± 0.3%), indicating that red-winged blackbirds had access to both aquatic and terrestrial habitats (online supplementary material Figure S2). We concluded the field portion of the study on July 31, 2017, when nestlings fledged from the last observed active nest of the season.

Collection of blood from red-winged blackbird nestlings to assess diet using stable isotope values and to assess Hg concentrations

Blood samples were used to (1) estimate nestling diet using stable isotopes of carbon and nitrogen and (2) assess MeHg contamination of nestlings. We attempted to collect blood samples at two time points between hatching and fledging from each nestling so that the values would be representative of the nestling period (which can last 11–14 days; Yasukawa & Searcy, 2020). The purpose of this approach was to reduce the influence of maternally transferred Hg, which is greatest immediately after hatching, and the effects of cessation of feather production, which occurs at fledging (Ackerman et al., 2011). Because we found no evidence of biologically meaningful age-related changes in isotope values or Hg concentrations in nestling blood (online supplementary material Figure S3) and our goal was not to evaluate age-related changes, we averaged isotope and Hg concentration data collected from the two time points.

From May 8 to July 31, 2017, we collected blood samples from each nestling in a nest (2.7 ± 0.8 nestlings per nest [mean ± SD]; range, 1–4 nestlings per nest). Blood samples were collected from 240 nestlings, with 184 nestlings sampled on two dates. The first sampling day ranged from 3 to 9 days posthatch with a mean sampling day of 5.0 ± 0.7 (mean ± SD) days posthatch. The second sampling day ranged from 6 to 11 days posthatch with a mean sampling day of 8.3 ± 0.7 days posthatch. Whenever possible, nest monitoring ended by day 10 posthatch to decrease the risk of premature fledging. On the first sampling date, we applied a unique color pattern to the legs of each nestling using permanent markers to allow for future identification. On the second sampling date, we banded each nestling with a United States Geological Survey band.

Blood samples were collected from the brachial vein with a sterile disposable 26-gauge needle and heparinized microcapillary tubes (Gillet & Seewagen, 2014). We collected approximately 50–150 µl of blood, < 1% of the nestling's body weight (Gaunt et al., 1997). Blood samples were placed on ice in the field, and then frozen at -20°C in the laboratory prior to analysis. To prepare blood samples for stable isotope analysis, a small volume (0.25–1.5 mg dry wt) of whole blood was dried on a precombusted glass fiber filter for 48 hr in a 60°C drying oven.

Nestlings within a nest are not independent; therefore, our goal was to produce a single carbon and nitrogen stable isotope value and average MeHg concentration for each nest ($n = 88$). To reduce costs associated with stable isotope analyses, one nestling per nest was selected at random and sampled 1–2 times, and the stable isotope values were averaged to produce one carbon and nitrogen stable isotope value per nestling. These stable isotope values from a single nestling were used to represent the isotope values of the nest. For MeHg, each nestling was sampled 1–2 times and the MeHg concentrations were averaged to produce one MeHg concentration per nestling. Next, the MeHg concentrations for all nestlings in a nest were averaged to produce a nest average.

Collection of emergent aquatic insects, spiders, and terrestrial insects

We collected representative taxa of three groups of arthropods (emergent aquatic insects, spiders, and terrestrial insects), which we confirmed were consumed by nestling red-winged blackbirds at our study site using an analysis of nestling fecal sacs (online supplementary material Table S2). Arthropods were collected May 19, May 30, June 8, and June 29, 2017, along 12 different sampling transects (~200–400 m in length) located throughout the pond facility and all within the potential foraging areas of the adult red-winged blackbirds nesting at the pond facility (online supplementary material Figure S2). Arthropods were collected from the ground and vegetation with nets or by hand and preserved in 95% ethanol in the field.

We collected 16 taxa of arthropods. Emergent aquatic insects included adult damselflies (Odonata: Zygoptera) and dragonflies (Odonata: Anisoptera: Libellulidae and Aeshnidae); spiders (Araneae) including jumping spiders (Salticidae), long-jawed orb-weavers (Tetragnathidae: *Tetragnatha sp.*), lynx spiders (Oxyopidae), and wolf spiders (Lycosidae); and terrestrial insects included caterpillars (Lepidoptera), crickets (Orthoptera: Grylloidea), grasshoppers (Orthoptera: Caelifera), ladybugs (Coleoptera: Coccinellidae), leaf beetles (Coleoptera: Chrysomelidae), leafhoppers (Hemiptera: Cicadellidae), sharpshooters (Hemiptera: Cicadellidae: Proconiini), terrestrial flies (Diptera: Calyptratae), and weevils (Coleptera: Curculionidae). On average, we collected 8.0 ± 8.3 (mean ± SD) individual emergent aquatic insects per taxa, 29.5 ± 7.3 individual spiders per taxa, and 43.7 ± 36.4 individual terrestrial insects per taxa from each sampling transect. Arthropods were pooled by taxon and sampling transect across sampling dates into 135 composite samples ($n = 27$, 27, and 81 composite samples for emergent aquatic insects, spiders, and terrestrial insects, respectively [online supplementary material Table S3]). Arthropod composite samples were dried at 60°C for 72 hr and then homogenized to a fine powder using a clean mortar and pestle or a ball mill.

Stable isotope analysis and dietary mixing model

To estimate the proportion of emergent aquatic insects, spiders, and terrestrial insects in diets of nestling red-winged blackbirds, we determined bulk carbon and nitrogen stable isotopes values ($\delta^{13}C$ and $\delta^{15}N$) of whole blood from nestling red-winged blackbirds and arthropods and modeled diet with a Bayesian inference stable isotope dietary mixing model. Whole blood from nestlings dried on precombusted glass fiber filters and dried ground arthropod samples were encased in tin capsules before analysis at the University of California, Davis Stable Isotope Facility using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. Carbon and nitrogen isotope values are reported as:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$$

where R is $^{13}C/^{12}C$ for $\delta^{13}C$ and $^{15}N/^{14}N$ for $\delta^{15}N$. Standards for $\delta^{13}C$ and $\delta^{15}N$ were Vienna Pee Dee Belemnite and atmospheric N_2, respectively. Reference samples and duplicate samples were used for quality assurance. During analysis, samples were interspersed with several replicates of four different laboratory reference materials (glutamic acid, nylon 6, bovine liver, and enriched alanine) that had been previously calibrated against international reference materials (https://stableisotopefacility.ucdavis.edu/carbon-and-nitrogen-solids). The average recovery percentage of the four reference materials were 100.0% (range, 99.5%–100.1%; $n = 144$) and 99.9% (range, 99.8%–101.3%; n = 171) for $\delta^{13}C$ and $\delta^{15}N$, respectively. The mean relative percent difference for duplicate samples was 0.21% (range, 0.04%–0.60%; $n = 7$) and 0.93% (range, 0.21%–2.19%; $n = 7$) for $\delta^{13}C$ and $\delta^{15}N$, respectively.

The Bayesian inference stable isotope dietary mixing model used the individual stable isotope values of nestling red-winged blackbirds ($n = 88$) and their potential arthropod prey items ($n = 135$ composite samples) determined in the present study to estimate nestling red-winged blackbird diet. Each potential arthropod prey item was assigned to one of three dietary sources (i.e., end-members) in our model; emergent aquatic insects ($n = 3$ taxa), spiders ($n = 4$ taxa), or terrestrial insects ($n = 9$ taxa); see *Collection of emergent aquatic insects, spiders and terrestrial insects* section above for a list of taxa in each group. The Bayesian inference stable isotope dietary mixing model uses isotope trophic enrichment factors from the literature (Caut et al., 2009) to generate posterior probability distributions of the proportional contributions of emergent aquatic insects, spiders, and terrestrial insects to red-winged blackbird diets (Parnell et al., 2010). Posterior probability distributions were then used to generate the mean proportion of each prey type in nestling red-winged blackbird diets.

Bayesian inference stable isotope dietary mixing model analysis was performed in the R package MixSIAR (version 3.1.12). Mean (± SD) trophic enrichment factors used in models were 0.59 ± 1.01‰ and 2.37 ± 0.62‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Caut et al., 2009). Model fitting was conducted with uninformed priors and a process × residual error structure via Markov Chain Monte Carlo (MCMC) simulations, which produces likely values of the proportional contribution of emergent aquatic insects, spiders, and terrestrial insect prey to the diet. The model was run with three MCMC chains (chain length = 1,000,000, with a 500,000 burn-in, and thinned by 500). Chain convergence was assessed with the Gelman-Rubin and Geweke diagnostics.

Total Hg analysis of nestling blood and arthropod prey

Whole blood samples from nestlings and dried ground arthropod samples were analyzed for total Hg (MeHg + inorganic Hg) with a Milestone DMA-80 Direct Analyzer, which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy (United States Environmental Protection Agency [USEPA], 1998b). Reference samples (National Research Council of Canada Institute for National Measurement Standards) and duplicate samples were used for quality assurance. Reference samples (DORM-4, fish protein; Willie et al., 2012) were analyzed every 10 samples, and the average recovery percentage was 103.7% (range, 93.2%–110.6%; $n = 97$). Duplicate samples were analyzed every 20 samples, and the mean relative percent difference was 3.4% (range, 0.1%–9.6%; $n = 32$). The method detection limit, calculated as 3.14 times the SD of 7 low-level samples, was 0.11 ng total Hg (or ~3.7 ng/g dry wt for a 30 mg sample). Eleven terrestrial insect samples were below the method detection limit and total Hg concentrations for these samples were estimated as 50% of the detection limit.

MeHg analysis and estimation: arthropods

The percentage of total Hg concentration that is MeHg in arthropods can be highly variable, in some studies ranging from 6% to 88% depending on taxa (Ortega-Rodriguez et al., 2019). We had previously determined the percentage of total Hg that was MeHg at our study site for 13 of the 16 taxa collected in the present study (Ortega-Rodriguez et al., 2019; Speir et al., 2014). Therefore, we used the taxon-specific percentage of MeHg determined in these previous studies to estimate MeHg concentrations from measurements of total Hg concentrations for these 13 taxa (online supplementary material Table S3). However, for three of the terrestrial insect taxa (leaf beetles, terrestrial flies, and weevils) that we collected in the present study, the percentage of total Hg that is MeHg had not been previously determined at our study site. Therefore, we analyzed MeHg concentrations in a subset of samples from these three taxa to determine the percentage of total Hg concentration that was MeHg.

We analyzed MeHg at the Marine Ecotoxicology and Trophic Assessment Laboratory (METAL) at the University of Alaska Fairbanks according to previously published methods (Hannappel et al., 2021). Briefly, dried ground terrestrial insects were analyzed for MeHg using a Brooks Rand MERX-M Automated MeHg Analytical System, which uses purge and trap, gas chromatography, and cold vapor atomic fluorescence spectroscopy (USEPA, 1998a). Samples (~5 mg) were digested with 30% HNO_3 for 20 hr in a water bath at 65 to 70°C. Cooled digests were diluted with ultrapure water and stored in the dark at room temperature until analysis within 48 hr. To quantify MeHg, 100- to 200-μl aliquots of the sample digests were added to individual glass vials containing ultrapure water and acetate buffer. A 1% solution of sodium tetraethylborate in KOH was added to each vial, and the total volumes were adjusted to 40 ml with ultrapure water. Reference samples (DORM-4 and International Atomic Energy Agency, IAEA-86, human hair; Bleise et al., 2000) were digested and analyzed with the samples. Additional quality assurance included the analysis of check standards (10 pg MeHg), blank spikes (10 pg MeHg), matrix spikes (10 pg MeHg), duplicate samples, and reagent blanks. Reagent blanks consisted of 30% HNO_3 without the addition of a sample. All sample digests were analyzed in triplicate. Any samples with a coefficient of variation among triplicates > 15% were reanalyzed until < 15% was achieved. The mean value of triplicates was used for statistical comparisons. Mean recovery percentages for DORM-4 and IAEA-86 were 92.7% (range, 80.8%–103.0%; $n = 6$) and 106.3% (range, 84.7%–125.2%; $n = 7$), respectively. The mean recovery percentages of MeHg from check standards, blank spikes, and matrix spikes were 99.8% (range, 94.4%–114.4%; $n = 18$), 101.2% (range, 91.7%–117.2%; $n = 6$), and 98.4% (range, 90.0%–102.9%; $n = 6$), respectively. The mean relative percent difference between duplicates was 5.4% (range, 0%–10.8%). The mean mass of MeHg in digestion blanks was 0.05 pg (range, 0.0–0.1; $n = 10$). All samples were above the method detection limit of 0.25 pg MeHg, calculated by adding the mean of reagent blanks to 3× the SD of the same blanks.

MeHg estimation: red winged blackbird nestlings

Methyl mercury accounts for approximately 95% of the Hg species in bird blood (Evers & Clair, 2005). Therefore, we assumed that 95% of total Hg in bird blood was MeHg and corrected measurements of total Hg accordingly to estimate MeHg in the blood of nestling red-winged blackbirds.

Statistical analysis

Analysis of variance (ANOVA) was used to determine whether MeHg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values differed among arthropod groups (emergent aquatic insects, spiders, and terrestrial insects). All analyses were completed using SPSS Ver. 26, and statistical significance was determined at $p < .05$.

Results and discussion

We determined carbon and nitrogen stable isotope values of potential arthropod prey and nestling red-winged blackbird blood and used a mixing model to assess the proportion of arthropod groups in nestling red-winged blackbird diets. Average $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE) varied significantly between potential arthropod prey (ANOVA, $F_{2,132} = 58.0$, $p < .0001$ and $F_{2,132} = 31.7$, $p < .0001$, respectively) and both isotopes were most enriched in spiders ($\delta^{13}C = -21.9 \pm 0.5‰$; $\delta^{15}N = 7.5 \pm 0.2‰$), followed by emergent aquatic insects ($\delta^{13}C = -22.7 \pm 0.7‰$; $\delta^{15}N = 6.1 \pm 0.3‰$) and terrestrial insects ($\delta^{13}C = -24.1 \pm 0.3‰$; $\delta^{15}N = 5.1 \pm 0.2‰$; Figure 3). Average $\delta^{13}C$ and $\delta^{15}N$ values in red-winged blackbird nestlings were -23.3 ± 0.2‰ and 7.8 ± 0.2‰, respectively. Average $\delta^{13}C$ and $\delta^{15}N$ values of red-winged blackbird nestlings were

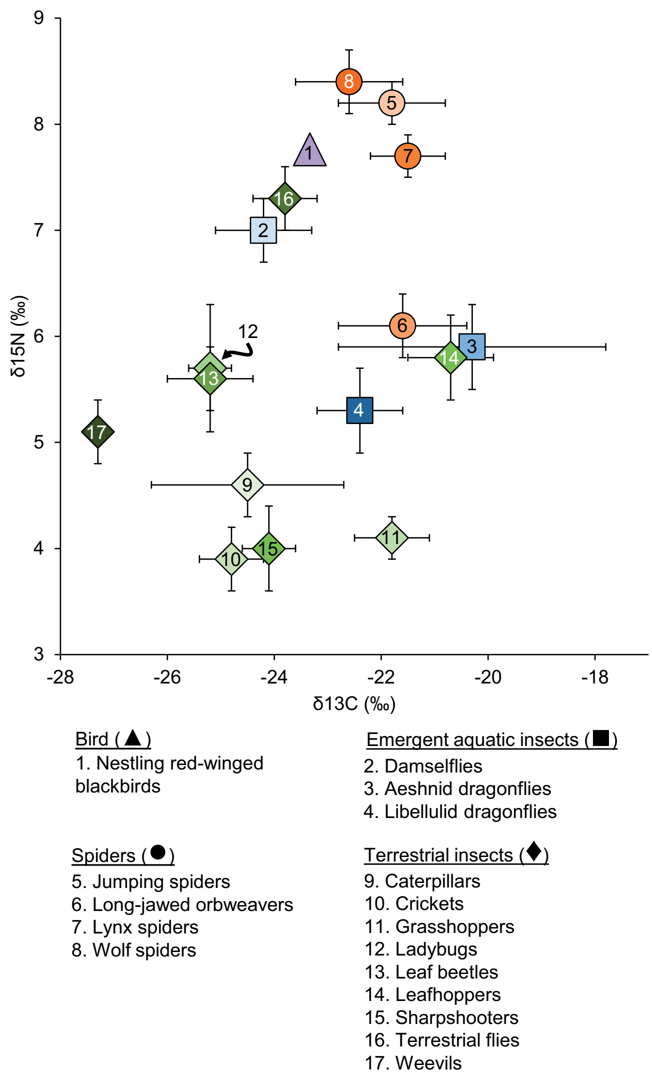

Figure 3. Mean (± *SE*) $\delta^{15}N$ vs $\delta^{13}C$ values for nestling red-winged blackbirds, emergent aquatic insects, spiders, and terrestrial insects. Sample sizes can be found in online supplementary material Table S3.

enriched by approximately 0.5–1‰ and approximately 2–3‰, respectively (i.e., amounts similar to presumed trophic enrichment factors), relative to several potential terrestrial insect prey items, suggesting terrestrial insect prey were important in nestling diet (Figure 3). Similarly, the stable isotope dietary mixing model indicated that the diets of nestling red-winged blackbirds was dominated by terrestrial insect prey items with the average proportion (mean ± *SE*) of emergent aquatic insects, spiders, and terrestrial insect prey in nestling diet estimated as 23.8% ± 3.1%, 6.4% ± 0.9%, and 69.7% ± 1.6%, respectively (Figure 4).

We assessed MeHg concentrations of potential arthropod prey items and the blood of red-winged blackbird nestlings. Average MeHg (mean ± *SE*) concentrations varied significantly between arthropod prey items (ANOVA, $F_{2,132} = 129.4$, $p < .0001$) and was highest in emergent aquatic insects (88.3 ± 9.1 ng/g dry wt), followed by spiders (67.6 ± 5.9 ng/g dry wt) and terrestrial insects (6.1 ± 1.0 ng/g dry wt; Figure 5). Methyl mercury concentrations in the blood of nestling red-winged blackbirds ranged from 5.1 to 85.4 ng/g wet weight, with an average (mean ± SE) value of 19.7 ± 1.6 ng/g wet weight (Figure 6). Methyl mercury concentrations in all nestlings were well below the lowest observed effects level for MeHg in bird blood (200 ng/g wet wt, Ackerman et al., 2016; Figure 6). Below this threshold, birds would not be expected to experience negative health effects from MeHg exposure.

In the absence of site-specific data, risk assessments based on “reasonable worst-case assumptions” of diet from the literature (i.e., that nestling red-winged blackbirds consume primarily emergent aquatic insects) have the benefit of producing conservative exposure estimates (European Food Safety Authority [EFSA] et al., 2023) but lack environmental realism (EFSA et al., 2023; Morrissey et al., 2024). A previous study at our study site (Williams et al., 2017) identified elevated concentrations of MeHg in emergent aquatic insects and a risk assessment model suggested that if diets of nestling red-winged blackbirds consisted primarily of emergent aquatic insects, nestlings could accumulate concentrations of MeHg in their blood that would put them at risk for negative health effects (Williams et al., 2017). We conducted this study to follow up on the findings of Williams et al. (2017) and found that emergent aquatic insects do not constitute a large proportion of nestling red-winged blackbird diet (< 25%) at our study site and that nestling red-winged blackbirds had not accumulated concentrations of MeHg in their blood that could put them at risk. Instead, the diet of nestling red-winged blackbirds at our study site was dominated by terrestrial insects, which have low concentrations of MeHg.

To our knowledge, this study is the first to simultaneously assess diet and MeHg concentrations in nestling red-winged blackbirds. Assessing diet and contaminant concentrations in nestlings in some species of free-living birds is challenging, due

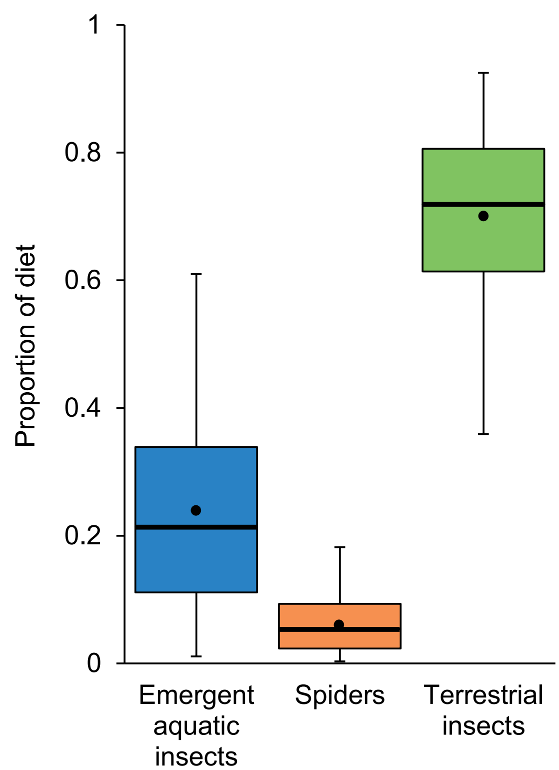

Figure 4. Proportion of emergent aquatic insects, spiders, and terrestrial insects in nestling red-winged blackbirds estimated from a $\delta^{15}N$ and $\delta^{13}C$ mixing model. Estimated proportional contribution of prey in nestling diets are represented by the mean (point), median (horizontal line), 25th and 75th percentile (boxes), and the 5th and 95th percentile (whiskers) posterior probability values.

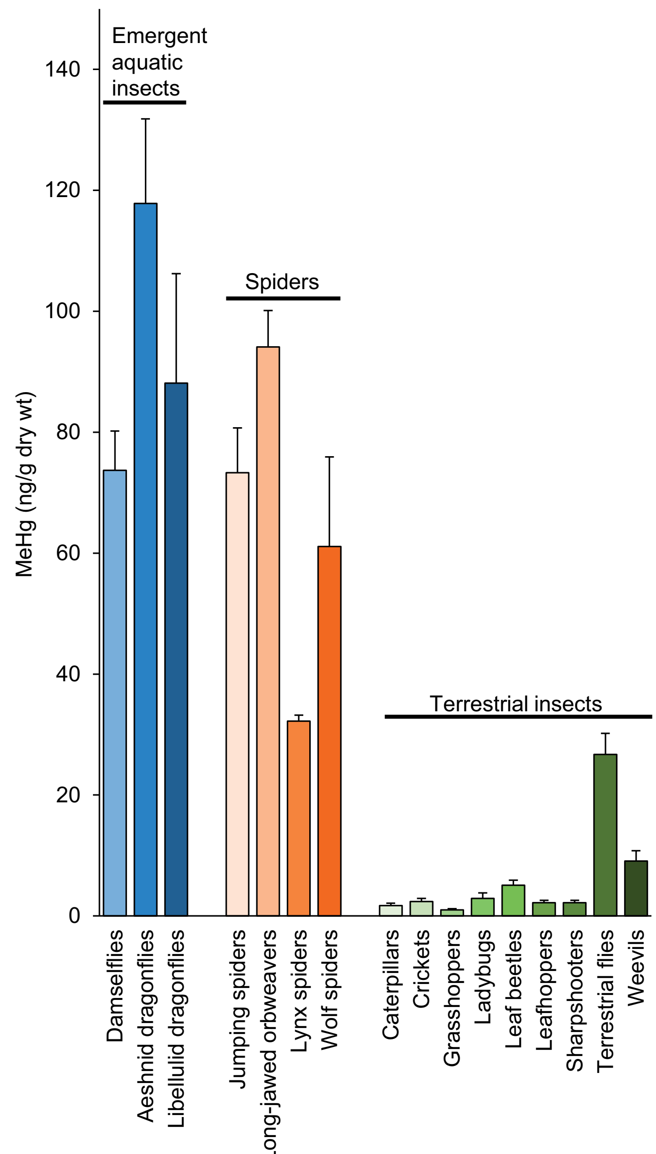

Figure 5. Mean (± *SE*) estimated methyl mercury (MeHg) in emergent aquatic insects, spiders and terrestrial insects, potential prey items in nestling red-winged blackbirds. Sample sizes can be found in online supplementary material Table S3.

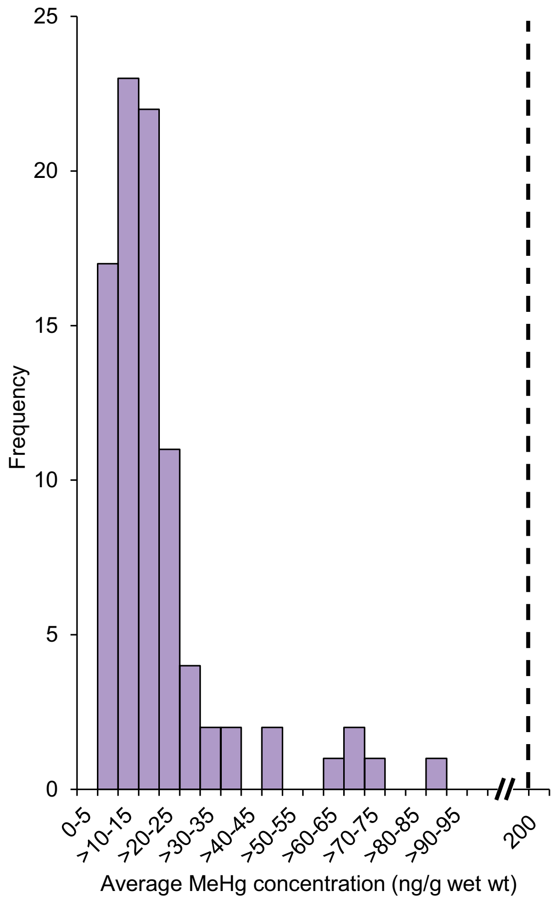

Figure 6. Frequency distribution of the average concentrations of estimated methyl mercury (MeHg) in the blood of the nestling red-winged blackbirds. Hashed vertical line represents the lowest observed effects level for MeHg in bird blood (Ackerman et al., 2016), below which birds would not be expected to experience negative health effects from MeHg.

to logistical difficulties in locating nests and collecting data from enough individuals to draw conclusions about a given study site. Despite these challenges, the study illustrates the necessity of collecting such data and suggests that caution must be used when interpreting risk assessment models for nestling red-winged blackbirds in the absence of site-specific estimates of diet. Because the diets of nestling red-winged blackbirds can consist of varying proportions of aquatic insects and spiders (online supplementary material Table S1), risk assessments based on estimates of diet from the literature that indicate nestlings might be at risk from Hg contamination (Williams et al., 2017) could overestimate risk and must be followed up with studies to assess diet and/or the actual level of contamination. Because the diets of other songbirds that nest in or near wetlands or riparian forests are also known to be composed of varying proportions of emergent aquatic insects and riparian spiders (e.g., tree swallows (*Tachycineta bicolor*), (McCarty & Winkler, 1999; Mengelkoch et al., 2004; online supplementary material Table S1), we hypothesize this caveat applies to a variety of songbird species, not just red-winged blackbirds. The results of the study demonstrate the importance of integrating the fields of toxicology and ecology for a more complete understanding of environmental Hg contamination and the risks Hg poses to wildlife (Chapman, 2002; Chumchal & Drenner, 2020; Morrissey et al., 2024; Schiesari et al., 2018).

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

Data are freely available from the Texas Christian University Scholarly Works repository (https://doi.org/10.18776/tcu/data/27013).

Author contributions

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Conflicts of interest

None declared.

Disclaimer

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References

Aagaard, A., Berny, P., Chaton, P., Antia, A. L., McVey, E., Arena, M., Fait, G., Ippolito, A., Linguadoca, A., Sharp, R., Theobald, A., & Brock, T.; European Food Safety Authority (EFSA). (2023). Risk assessment for birds and mammals. *EFSA Journal*, *21*, e07790.

Ackerman, J. T., Eagles-Smith, C. A., & Herzog, M. P. (2011). Bird mercury concentrations change rapidly as chicks age: Toxicological risk is highest at hatching and fledging. *Environmental Science & Technology*, *45*, 5418–5425.

Ackerman, J. T., Eagles-Smith, C. A., Herzog, M. P., Hartman, C. A., Peterson, S. H., Evers, D. C., Jackson, A. K., Elliott, J. E., Vander Pol, S. S., & Bryan, C. E. (2016). Avian mercury exposure and toxicological risk across western North America: A synthesis. *The Science of the Total Environment*, 568, 749–769.

Bleise, A. R., Heller-Zeisler, S. F., & Parr, R. M. (2000). *Reference material: IAEA-086 Methylmercury, total mercury and other trace elements in human hair*. International Atomic Energy Agency. https://nucleus.iaea.org/sites/AnalyticalReferenceMaterials/Shared%20Documents/ReferenceMaterials/TraceElements/IAEA-086/RS_IAEA-086.pdf

Brasso, R. L., & Cristol, D. A. (2008). Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). *Ecotoxicology (London, England)*, *17*, 133–141.

Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors (Δ15N and Δ13C): The effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46, 443–453.

Chapman, P. M. (2002). Integrating toxicology and ecology: Putting the "eco" into ecotoxicology. *Marine Pollution Bulletin*, *44*, 7–15.

Chumchal, M. M., Beaubien, G. B., Drenner, R. W., Hannappel, M., Mills, M. A., Olson, C. I., Otter, R. R., Todd, A. C., & Walters, D. M. (2022). Use of riparian spiders as sentinels of persistent and bioavailable chemical contaminants in aquatic ecosystems: A review. *Environmental Toxicology and Chemistry*, *41*, 499–514. https://doi.org/10.1002/etc5267

Chumchal, M. M., & Drenner, R. W. (2020). Ecological factors controlling insect-mediated methyl mercury flux from aquatic to terrestrial ecosystems: Lessons learned from mesocosm and pond experiments. *Contaminants and Ecological Subsidies: The Land-Water Interface*, 17–33.

Chumchal, M. M., Drenner, R. W., Greenhill, F. M., Kennedy, J. H., Courville, A. E., Gober, C. A., & Lossau, L. O. (2017). Recovery of aquatic insect-mediated methylmercury flux from ponds following drying disturbance. *Environmental Toxicology and Chemistry*, 36, 1986–1990.

Chumchal, M. M., Drenner, R. W., Hall, M. N., Polk, D. K., Williams, E. B., Ortega-Rodriguez, C. L., & Kennedy, J. H. (2018). Seasonality of dipteran-mediated methylmercury flux from ponds. *Environmental Toxicology and Chemistry*, *37*, 1846–1851.

Cristol, D. A., Brasso, R. L., Condon, A. M., Fovargue, R. E., Friedman, S. L., Hallinger, K. K., Monroe, A. P., & White, A. E. (2008). The movement of aquatic mercury through terrestrial food webs. *Science (New York, N.Y.)*, 320, 335–335. https://doi.org/10.1126/science.1154082

Driscoll, C. T., Mason, R. P., Chan, H. M., Jacob, D. J., & Pirrone, N. (2013). Mercury as a global pollutant: Sources, pathways, and effects. *Environmental Science & Technology*, *47*, 4967–4983.

Evers, D. C., & Clair, T. A. (2005). Mercury in northeastern North America: A synthesis of existing databases. *Ecotoxicology (London, England)*, *14*, 7–14.

Gann, G. L., Powell, C. H., Chumchal, M. M., & Drenner, R. W. (2015). Hg-contaminated terrestrial spiders pose a potential risk to songbirds at Caddo Lake (Texas/Louisiana, USA): Hg-contaminated terrestrial spiders and potential risk to songbirds. *Environmental Toxicology and Chemistry*, *34*, 303–306. https://doi.org/10.1002/etc2796

Gaunt, A. S., Oring, L. W., Able, K., Anderson, D., Baptista, L., Barlow, J., & Wingfield, J. (1997). *Guidelines to the use of wild birds in research*. Washington DC: The Ornithological Council.

Gillet, A.-M. T. Y., & Seewagen, C. L. (2014). Mercury exposure of a wetland songbird, *Agelaius phoeniceus*, in the New York metropolitan area and its effect on nestling growth rate. *Environmental Monitoring and Assessment*, *186*, 4029–4036.

Hannappel, M. P., Chumchal, M. M., Drenner, R. W., Kennedy, J. H., Barst, B. D., Castellini, J. M., Nolan, A. R., Willoughby, F. M., & Trauffler, L. P. (2021). Effect of body size on methyl mercury concentrations in shoreline spiders: Implications for their use as sentinels. *Environmental Toxicology and Chemistry*, *40*, 1149–1154. https://doi.org/10.1002/etc4964

Jackson, A. K., Eagles-Smith, C. A., & Robinson, W. D. (2021). Differential reliance on aquatic prey subsidies influences mercury exposure in riparian arachnids and songbirds. *Ecology and Evolution*, *11*, 7003–7017.

Lepak, J. M., Hooten, M. B., Eagles-Smith, C. A., Tate, M. T., Lutz, M. A., Ackerman, J. T., Willacker, J. J., Jackson, A. K., Evers, D. C., Wiener, J. G., Pritz, C. F., & Davis, J. (2016). Assessing potential health risks to fish and humans using mercury concentrations in inland fish from across western Canada and the United States. *The Science of the Total Environment*, *571*, 342–354.

McCarty, J. P., & Winkler, D. W. (1999). Foraging ecology and diet selectivity of tree swallows feeding nestlings. *The Condor*, *101*, 246–254.

Mengelkoch, J. M., Niemi, G. J., & Regal, R. R. (2004). Diet of the nestling tree swallow. *The Condor*, *106*, 423–429.

Morrissey, C., Fritsch, C., Fremlin, K., Adams, W., Borgå, K., Brinkmann, M., Eulaers, I., Gobas, F., Moore, D. R. J., van den Brink, N., & Wickwire, T. (2024). Advancing exposure assessment approaches to improve wildlife risk assessment. *Integrated Environmental Assessment and Management*, 20, 674–698.

Ortega-Rodriguez, C. L., Chumchal, M. M., Drenner, R. W., Kennedy, J. H., Nowlin, W. H., Barst, B. D., Polk, D. K., Hall, M. N., Williams, E. B., Lauck, K. C., Santa-Rios, A., & Basu, N. (2019). Relationship between methylmercury contamination and proportion of aquatic and terrestrial prey in diets of shoreline spiders. *Environmental Toxicology and Chemistry*, 38, 2503–2508. https://doi.org/10.1002/etc4579

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: Coping with too much variation. *PloS One*, 5, e9672.

Schiesari, L., Leibold, M. A., & Burton, G. A. Jr, (2018). Metacommunities, metaecosystems and the environmental fate of chemical contaminants. *Journal of Applied Ecology*, 55, 1553–1563.

Speir, S. L., Chumchal, M. M., Drenner, R. W., Cocke, W. G., Lewis, M. E., & Whitt, H. J. (2014). Methyl mercury and stable isotopes of nitrogen reveal that a terrestrial spider has a diet of emergent aquatic insects: Methyl mercury and stable isotopes in emergent insects and spiders. *Environmental Toxicology and Chemistry*, 33, 2506–2509. https://doi.org/10.1002/etc2700

Tweedy, B. N., Drenner, R. W., Chumchal, M. M., & Kennedy, J. H. (2013). Effects of fish on emergent insect-mediated flux of methyl mercury across a gradient of contamination. *Environmental Science & Technology*, *47*, 1614–1619. https://doi.org/10.1021/es303330m

United States Environmental Protection Agency. (1998a). *Method 1630, Methyl mercury in water by distillation, aqueous ethylation,* *purge and trap, and CVAFS*. US Environmental Protection Agency.

United States Environmental Protection Agency. (1998b). Method 7473 (SW-846): Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry.

Wiener, J. G., Krabbenhoft, D. P., Heinz, G. H., & Scheuhammer, A. M. (2003). Ecotoxicology of mercury. *Handbook of Ecotoxicology*, 2, 409–463.

Williams, E. B., Chumchal, M. M., Drenner, R. W., & Kennedy, J. H. (2017). Seasonality of odonate-mediated methylmercury flux from permanent and semipermanent ponds and potential risk to red-winged blackbirds (*Agelaius phoeniceus*). *Environmental Toxicology and Chemistry*, 36, 2833–2837.

Willie, S. N., Brophy, C., Clancy, V., Lam, J., Sturgeon, R., & Yang, L. (2012). *DORM-4: Fish protein certified reference material for trace metals*. National Research Council Canada. https://doi.org/10.4224/crm.2012.dorm-4

Yasukawa, K., & Searcy, W. A. (2020). Red-winged blackbird (*Agelaius phoeniceus*), version 1.0. In P. G. Rodewald (Ed.), *Birds of the World*. Cornell Lab of Ornithology. https://doi.org/10.2173/bow.rewbla.01

Environmental Toxicology and Chemistry, 2025, 44, 59–67
https://doi.org/10.1093/etojnl/vgae018
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